JB F41 Poms

Suresh Gopalan and Sheng Yang He
MSU-DOE Plant R search Laboratory, Michigan State University, East Lansing

Bacterial Genes Involved in the Elicitation of Hypersensitive Response and Pathogenesis

Intensive molecular genetic studies undertaken in the past 10 years have started to solve many of the puzzles in the area of compatibility and incompatibility between plants and bacterial pathogens. These studies have provided answers to some of the most fundamental questions in plant pathology: What bacterial genes are involved in the establishment of compatibility or incompatibility between plants and necrogenic bacteria? What traits distinguish plant-pathogenic bacteria from saprophytic bacteria? Are these genes and traits common in seemingly very diverse groups of plant-pathogenic bacteria, from soft-rot erwinias to local lesion-forming pseudomonads? In this article, we will discuss some recent advances in understanding the compatibility or incompatibility between plants and necrogenic bacteria (bacteria that cause tissue necrosis). The potential application of these advances to disease management will be addressed briefly. Interested readers should consult other recent reviews (6,8,45,50) for a more technical discussion on this topic.

Plant–Bacteria Interactions: Incompatible vs. Compatible

Plant-pathogenic bacteria cause devastating diseases on many important crop plants. Some bacteria, such as *Agrobacterium tumefaciens*, cause tissue deformation (tumors) by altering hormone balance in infected plant tissues. Other bacteria cause wilt or soft rot by interfering with the function of the plant vascular system or by disintegrating plant tissues, respectively. Many pathovars of *Pseudomonas syringae* and *Xanthomonas campestris* cause local lesions on various plant tissues. Disease symptoms caused by most plant-pathogenic bacteria involve plant cell death. In this article, only necrogenic bacteria will be discussed. Therefore, gall-forming *A. tumefaciens* and other bacteria that do not cause necrosis will not be addressed.

Plant–bacteria interactions can be generally classified as compatible or incompatible interactions. In a compatible interaction, a susceptible host plant is infected by a virulent (or compatible) bacterium, resulting in the multiplication and spread of the bacterium in infected plant tissues and the appearance of disease symptoms. In an incompatible interaction, an avirulent (or incompatible) bacterium attempts to infect a resistant host plant or a nonhost plant, but the multiplication and spread of the bacterium are severely restricted. A hallmark of many incompatible interactions is the occurrence of rapid plant cell death at or near the attempted infection site, a phenomenon known as the hypersensitive response (HR; 16,29). That is, although an avirulent bacterium is unable to cause typical spreading disease symptoms in a resistant host or nonhost plant, it is able to elicit localized plant cell death. The HR is associated with a wide array of defense responses that may inhibit further pathogen invasion, including synthesis of antimicrobial compounds, induction of plant defense genes, and strengthening of the plant cell wall by rapid cross-linking of cell wall components (10,32).

Although a true plant-pathogenic bacterium can elicit a dramatic plant response—either disease or resistance—in a healthy plant with the appropriate genetic background, saprophytic bacteria or bacteria that are pathogenic on organisms other than higher plants are generally unable to initiate any interactions in plants. Of 1,600 known species of bacteria, only about 80 species have been found to cause plant disease (1). What are the features that distinguish plant-pathogenic bacteria from other types of bacteria? Taxonomic differences give no clue to the differences in pathogenicity. For example, *Erwinia amylovora*, the bacterium that causes fire blight, is taxonomically more closely related to the human pathogens *Escherichia coli* and *Yersinia* spp. than to another common plant pathogen, *P. syringae*.

Genes Controlling Compatibility Between Plants and Bacteria

In the early 1980s, a number of researchers started to use transposon-mediated mutagenesis, a technique developed in the study of *E. coli*, to reveal bacterial genes that play important roles in various plant–bacteria interactions. A transposon is a mobile DNA element that can hop in and out of the bacterial chromosome. When a transposon hops into a gene on the chromosome, the gene is physically disrupted and cannot produce a functional product (Fig. 1). If the gene happens to be important in plant–bacterial interactions, the mutant bacterium carrying the disrupted gene will show a defect in initiating normal plant–bacterial interactions.

Using such a mutagenesis technique, Niepold et al. (35) and Lindgren et al. (33) identified clusters of bacterial genes, known as *hrp* (for HR and pathogenicity) genes, in the bean pathogens *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *phaseolicola*, respectively. Transposon-induced mutations in *hrp* genes were found to abolish the ability of *P. syringae* to elicit the HR in nonhost plants or to cause disease in host plants (33,35). *hrp* mutants behave very much like bacteria that have no apparent interactions with plants, such as *E. coli*. The identification of *hrp* genes suggested that the molecular mechanism(s) underlying bacterial pathogenicity and bacterial elicitation of plant disease resistance may involve the same bacterial genes.

hrp genes have been isolated from many plant-pathogenic bacteria and have been characterized most extensively from *P. s.* pv. *syringae*, *P. s.* pv. *phaseolicola*, *Pseudomonas solanacearum* (which causes wilt in many solanaceous plants), *Xanthomonas campestris* pv. *vesicatoria* (which causes bacterial spot on tomato and pepper), and *E. amylovora* (6,8,45). Isolation (cloning) of *hrp* genes was accomplished by inserting random genomic DNA fragments from a wild-type, plant-pathogenic bacterium into a cloning vector, followed by introduction of cloned DNA fragments into *hrp* mutants

Dr. He's address is: MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312; Phone: 517-353-9181, Fax: 517-353-9168, E-mail: hes@pilot.msu.edu

Publication no. D-1996-0313-04F

(Fig. 1). If a cloned DNA fragment carries a wild-type copy of the mutated *hrp* gene in an *hrp* mutant, it will produce a functional *hrp* gene product and therefore complement the mutated *hrp* gene located in the chromosome (Fig. 1). Surprisingly, the cloned *hrp* clusters from *P. s.* pv. *syringae* 61 and *E. amylovora* 321 enabled nonpathogens (e.g., *E. coli* or *Pseudomonas fluorescens*) to elicit the HR in plants (5,24). The functional cloning of these two *hrp* clusters in *E. coli* revealed that the minimum number of genes required for elicitation of the HR by plant-pathogenic bacteria is carried on a DNA fragment about 25 to 30 kb in length, a very small portion of the bacterial genome (which is normally about 4,000 to 5,000 kb).

DNA–DNA hybridization studies indicate that at least some *hrp* genes are similar among necrogenic bacteria belonging to different genera (*P. syringae*, *E. amylovora*, *Erwinia stewartii*, *P. solanacearum*, and *X. campestris*) (31). Recent DNA sequence studies confirm that many *hrp* genes cloned from diverse plant-pathogenic bacteria are homologous (23,46). Thus, *hrp* genes appear to be universal among diverse, necrosis-causing, gram-negative bacterial pathogens of plants.

Biochemical Functions of *hrp* Genes

The biochemical functions of *hrp* genes have remained a puzzle until recently. DNA sequencing has played a major role in the determination of many *hrp* gene functions. As will be discussed, many *hrp* genes have striking similarities with genes of known function. Figure 2 shows the gene organization and likely functions of *hrp* genes of *P. s.* pv. *syringae* (23). There are at least 25 *hrp* genes in this bacterium. Based on DNA sequence similarity to other known genes and subsequent biochemical and molecular characterization, we now know that *hrp* genes have at least three biochemical functions: gene regulation, protein secretion, and production of HR elicitor proteins.

1. Gene regulation. It was discovered that *hrp* genes either are not expressed or are expressed at very low levels (i.e., they make very low levels of protein products) when bacteria were grown in nutrient-rich bacteriological media, whereas they are highly expressed when bacteria are in the intercellular space (apoplast) of plant tissues (25,37,41,46,48,52,53). What conditions in plant tissues induce the expression of *hrp* genes, and how do bacteria detect these inducing conditions? Unlike viruses, nematodes, and many fungi, plant-pathogenic bacteria do not invade living plant cells. Therefore, signal exchanges between plant cells and bacteria must occur in (or through) the apoplast outside the plant cell. A number of laboratories have observed that induction of *P. syringae hrp* genes could be achieved by using artificial minimal media lacking complex nitrogen nutrients, indicating that lack of nutrients in the plant apoplast may be the signal for the induction of *hrp* genes (25,37,52,53). Specific compounds (e.g., sucrose and sulfur-containing amino acids) present in the plant apoplast may also serve as signals for the induction of *X. c.* pv. *vesicatoria hrp* genes (41). The induction of *hrp* genes in the nutrient-poor plant apoplast or in artificial minimal media indicates that *hrp* genes may be involved in bacterial nutrition in planta.

How do bacteria sense the plant apoplast environment? It was found that at least three of the 25 *hrp* gene products are involved in detection of the apoplast environment by *P. syringae*: *hrpL*, *hrpS*, and *hrpR* (18,51; Fig. 2). The *hrpS* and *hrpR* are among the first two *hrp* genes to be expressed once bacteria enter plant tissues (51,52). It has been hypothesized that the HrpS and HrpR proteins, once produced, bind to the "promoter" sequence of the *hrpL* gene to "promote" the production of the *HrpL* protein (51). Once the HrpL protein is produced, it activates promoters of other *hrp* genes and some bacterial avirulence (*avr*) genes, which determine gene-for-gene interactions between bacteria and plants (25,26,38,40,51; Fig. 3). Not all bacterial *avr* genes are regulated by *hrp* genes (30). Interestingly, *hrpS* and *hrpR* are similar in sequence to a family of bacterial proteins that regulate genes involved in diverse metabolic functions, including genes involved in nutrient transport and metabolism (18,51). The sequence similarity of *hrpS* and *hrpR* with gene regulators involved in nutrition appears to support the hypothesis that *hrp* genes are involved in bacterial nutrition in the nutrient-poor plant apoplast. This hypothesis is further supported by the observation that the expression of *hrp* genes can be turned off by complex nitrogen sources, tricarboxylic acid cycle intermediates, high osmolarity, and neutral pH, some of which are characteristic of rich bacterial media (25,37,41,46,52,53).

An *hrpS* homolog has been found in a very different bacterium, *E. amylovora* (S. V. Beer, *personal communication*). In *P. solanacearum*, a different *hrp* gene (*hrpB*) was found to be involved in the detection of the plant apoplast (15). Thus, different bacteria may or may not use the same mechanism to detect the apparently similar environment in the plant apoplast.

2. Protein secretion. One surprising finding from the sequence analysis of *hrp* genes was that many *hrp* genes show striking similarities to those involved in the secretion of proteinaceous virulence factors in human and animal pathogenic bacteria (12,17,22,39,46). Most plant-pathogenic

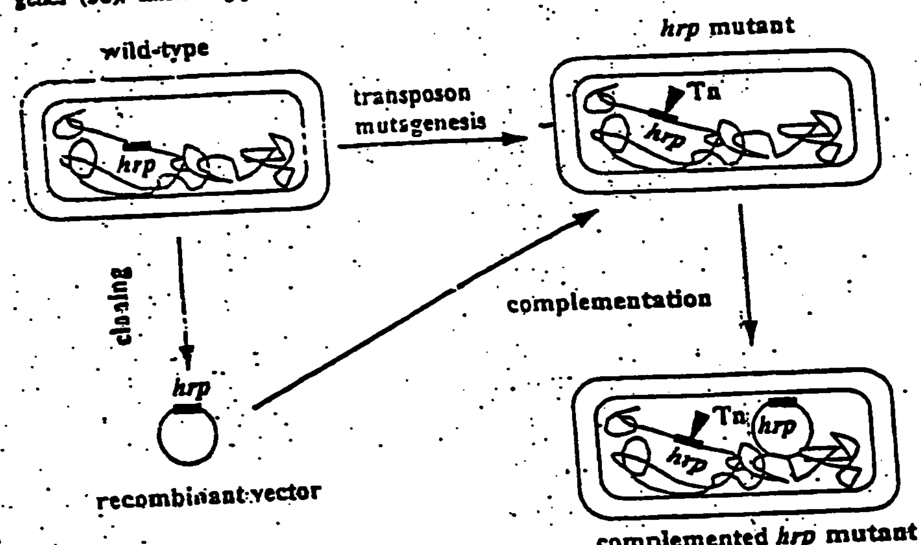

Fig. 1. Diagram of molecular techniques commonly used in the cloning of *hrp* genes. A wild-type bacterium is mutagenized by random insertion of a transposon (Tn) into its genome. When a transposon inserts into a wild-type *hrp* gene (in red), it physically disrupts the *hrp* gene (in green). The transposon-inserted *hrp* gene cannot produce a functional product, and the resulting bacterium is called a *hrp* mutant. The *hrp* mutant can no longer induce the hypersensitive response (HR) in resistant plants or cause disease in susceptible plants. To isolate (clone) the *hrp* gene identified by transposon mutagenesis, a gene library is established by inserting pieces of the wild-type genomic DNA into a cloning vector (indicated by a circle). The vector carrying foreign DNA inserts (recombinant vector) is then introduced into the *hrp* mutant. If a recombinant vector happens to carry a wild-type copy of the mutated *hrp* gene, it will produce a functional *hrp* gene product lacking in the *hrp* mutant, thus recovering the ability of the mutant to induce the HR in resistant plants and to cause disease in susceptible plants. The *hrp* mutant phenotype is therefore complemented by this recombinant vector.

bacteria that cause necrosis are gram-negative, that is, they have two cell membranes enveloping the cytoplasm. For any large molecule (e.g., a protein) to go through a lipid membrane, a special secretion apparatus or channel composed of many proteins must be assembled across both cell membranes. Gram-negative plant pathogenic bacteria are known to make several types of secretion apparatus. For example, *Erwinia chrysanthemi*, a bacterium that causes soft rot, makes one type of secretion apparatus for proteases and another for plant cell wall–degrading enzymes (21,39). Both types of secretion apparatus are widely conserved among many other bacteria, including human pathogens such as *E. coli* and *Pseudomonas aeruginosa* (21,39). The *hrp* genes were found to specify a third type of secretion apparatus, the Hrp secretion apparatus, which appears to be similar to the one discovered in several human-pathogenic bacteria, including *Yersinia* spp. (12,17,22,39,46). Interestingly, although the regulatory *hrp* genes in different bacteria may be different (*hrpS*, *hrpR*, and *hrpL* in *P. syringae* versus *hrpB* in *P. solanacearum*), most *hrp* genes involved in the assembly of the Hrp secretion apparatus are similar among diverse plant-pathogenic bacteria. This suggests that although different bacteria may detect the plant apoplast environment in their own unique ways, they nevertheless produce similar types of protein secretion apparatus.

3. Production of elicitor proteins. The discovery of the novel Hrp secretion apparatus raised an immediate question: What are the proteins that traverse it? Since *hrp* genes are essential for bacteria, both to elicit the plant HR and to cause disease, it was expected that some of the proteins that traverse the Hrp secretion apparatus may be elicitors of plant HR and that others may be involved in causing necrosis during pathogenesis. Wei et al. (47) first provided evidence that one of the *E. amylovora hrp* genes (*hrpN*) encodes a proteinaceous elicitor (harpin). Harpin elicits HR necrosis when injected into the apoplast of appropriate plants (47). Although no *hrpN* gene homolog could be found in *P. syringae*, another proteinaceous HR elicitor (harpin$_{Pss}$) was identified and was shown to be encoded by a different *hrp* gene, *hrpZ* (20,36). Furthermore, harpin$_{Pss}$ was the first extracellular protein shown to be secreted via the Hrp secretion apparatus (20). A third bacterial protein elicitor of the HR was identified in *P. solanacearum* and was named PopA1 (2). The *E. amylovora* harpin, *P. s.* pv. *syringae* 61 harpin$_{Pss}$, and *P. solanacearum* PopA1, although largely dissimilar in primary sequences, share similar properties that may be important in their HR elicitor activities. They are all heat stable, glycine rich, and hydrophilic. Homologs of *E. amylovora* harpin and *P. s.* pv. *syringae* 61 harpin$_{Pss}$ have been identified in other pathogenic strains that belong to the genus *Erwinia* and the species *P. syringae*, respectively (4,20). Thus, at least three proteins that traverse the Hrp secretion apparatus of three diverse bacteria elicit the HR.

The Search for Proteins that Traverse the Hrp Apparatus

As mentioned earlier, bacterial mutants defective in the Hrp secretion apparatus are unable to elicit the HR in resistant plants and to cause disease in susceptible plants. The question is, how many proteins are secreted via the Hrp secretion apparatus? If harpins and PopA are the only proteins that traverse the Hrp secretion apparatus, then mutations in the genes that make harpins and PopA would also eliminate the ability of bacteria to elicit the HR in resistant plants and to cause disease in host plants. However, if there are other pathogenicity- or HR-related proteins secreted via the Hrp apparatus, mutations in only harpin- or PopA-encoding genes would not completely abolish the ability of bacteria to elicit the HR in resistant plants or to cause disease in host plants. Wei et al. (47) reported that mutations in the gene coding for harpin of *E. amylovora* destroyed the ability of the bacteria both to trigger the HR in resistant nonhost tobacco and to cause disease in susceptible pear fruits. Mutations in the gene coding for harpin$_{Ech}$ of *E. chrysanthemi* prevented the bacterium from triggering the HR in the nonhost tobacco and reduced the ability of the bacterium to initiate disease lesions in host plants (4). In the case of harpin$_{Pss}$ of *P. syringae*, mutation analysis has been complicated by the complex gene structure and organization surrounding the *hrpZ* gene. Conclusive data regarding the role of harpin$_{Pss}$ in plant–*P. syringae* interactions are yet to be shown. PopA1 was shown to

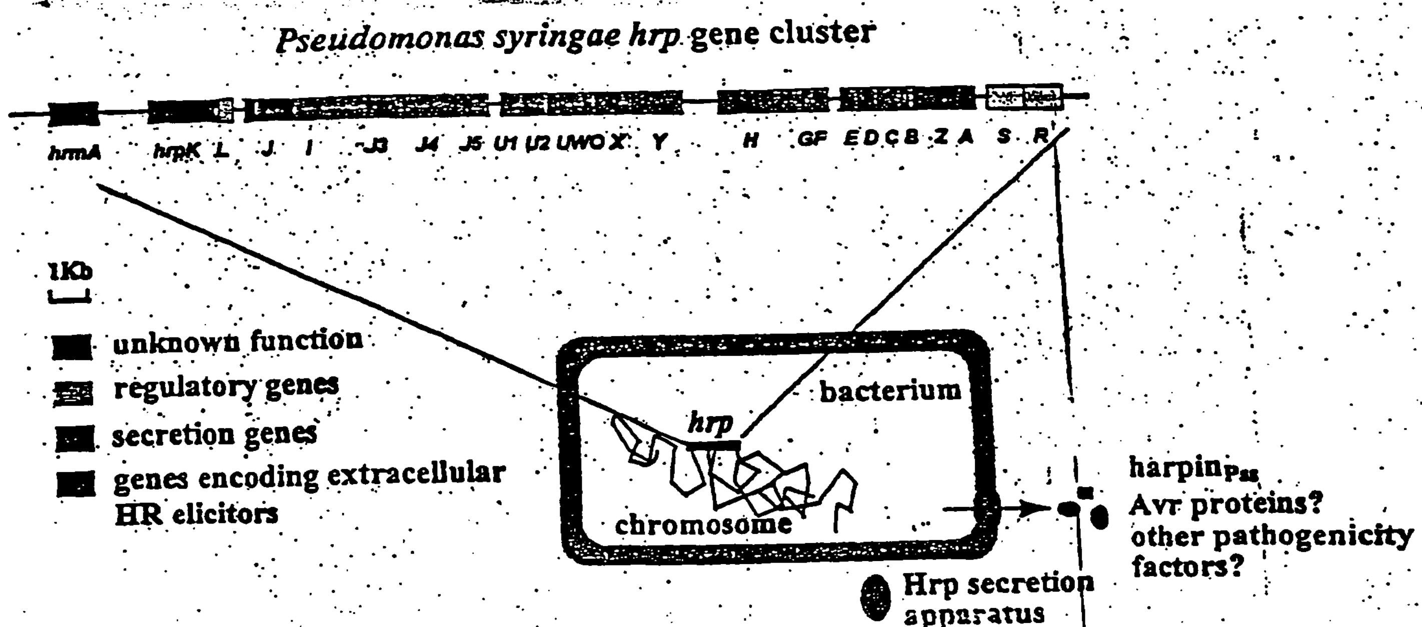

Fig. 2. *hrp* genes of *Pseudomonas syringae*. There are at least 25 *hrp* genes (*hrpA* to *hrpZ*) in *P. syringae*. *hrpS*, *hrpR*, and *hrpL* (in yellow) are involved in the detection of the plant apoplast environment and in the activation of all other *hrp* genes, *avr* genes, and possibly other pathogenicity-related genes. Most other *hrp* genes (in red) are involved in the assembly of the Hrp secretion apparatus in the bacterial envelope, through which travels a newly discovered class of bacterial virulence/avirulence proteins (in green), including HrpZ.

be dispensable for pathogenicity of *P. solanacearum* in the susceptible host plant, tomato, or for HR elicitation in the nonhost plant, tobacco (2), indicating that there must be other HR-elicitors and pathogenicity factors that traverse the Hrp secretion apparatus in this bacterium. Further examination indicated that PopA1 may function as an avirulence gene, mediating gene-for-gene interaction in certain Petunia–*P. solanacearum* interactions (2,45). Thus, the Hrp secretion apparatus in each bacterium may secrete a different number of proteins. Identification of other proteins that traverse the Hrp secretion apparatus is now an active research area and is essential for a complete understanding of *hrp*-mediated plant–bacterial interactions.

Factors Modifying *hrp* Gene-Mediated Compatibility

Two broad classes of bacterial genes may superimpose their functions on the *hrp* gene-mediated compatibility or incompatibility between plants and bacteria: *avr* genes and various virulence genes. The *avr* genes mediate genotype-specific incompatibility in resistant host plants. Virulence genes promote the production of disease symptoms and are usually needed for the full virulence of bacteria.

Bacterial *avr* Genes

Flor (14) formulated the gene-for-gene hypothesis in his work on flax–flax rust interactions. Flor hypothesized that the resistance of a given flax cultivar to a given fungal race is the result of the interaction between a fungal *avr* gene and a corresponding flax resistance gene. Therefore, the interactions between the plant's resistance genes and the pathogen's *avr* genes would limit the host range of the pathogen. Staskawicz et al. (44) first cloned an *avr* gene from a soybean bacterial pathogen, *Pseudomonas syringae* pv. *glycinea*, and showed that the cloned *avr* gene could convert virulent *P. s.* pv. *glycinea* strains that cause disease into avirulent strains that elicit the HR in certain soybean cultivars carrying the corresponding resistance genes, thus validating the role of *avr* genes in controlling host range. Since then, numerous *avr* genes have been cloned from plant-pathogenic bacteria (27). Several plant resistance genes have also been cloned using molecular genetic approaches (e.g., 34,43).

What is the relationship between the *avr* genes and *hrp* genes, both of which are involved in eliciting the HR? Several laboratories have observed that *avr* genes cannot trigger the genotype-specific HR in *hrp* mutants, i.e., *avr* genes depend on functional *hrp* genes for expressing their phenotype (25,26,28,38,40). There are several ways of explaining such dependence (Fig. 4). One possibility is that Avr proteins are dependent on the Hrp secretion apparatus for secretion. Alternatively, Avr function requires a prior plant response elicited by the *hrp*-controlled extracellular factors (such as harpins). A third possibility is that Avr proteins, with no HR-eliciting activity by themselves, cause the cultivar-specific HR by either covalently modifying harpins or modulating the expression of harpins in a plant resistance gene-dependent manner yet to be understood. Finally, it is also possible that Avr proteins are secreted directly into the plant cell with the help of harpins, assuming that receptors for Avr proteins are inside the plant cell. Studies are being carried out to resolve these possibilities.

Bacterial Virulence Factors

The genetic diversity of plant-pathogenic bacteria is reflected in their ability to cause diverse disease symptoms ranging from soft rot to tissue necrosis to "wildfire." These diverse disease symptoms are likely the result of the action of several, sometimes unique, virulence factors produced by a given bacterium in addition to *hrp*-controlled pathogenicity factors. For example, research from many laboratories has shown that toxin production plays an important role in the formation of chlorosis and necrosis (3,19,49). Extracellular polysaccharides may be involved in the formation of water-soaking lesions (11,13) and in the production of wilt symptoms by clogging the plant vascular system (9). Plant cell wall–degrading enzymes are responsible for tissue disintegration and the appearance of the soft-rot symptom (7). Plant hormones produced by plant-pathogenic bacteria are involved in the induction of tissue deformation (42).

Both *hrp* genes and bacterial virulence factors are necessary for disease symptom production, but what is the relationship between them? A logical relationship would be that *hrp*-controlled extracellular factors are involved in obtaining nutrients in early stages of pathogenesis, whereas other virulence factors drive the initial compatible stage into a fully compatible one, leading to the production of various disease symptoms. At least two lines of

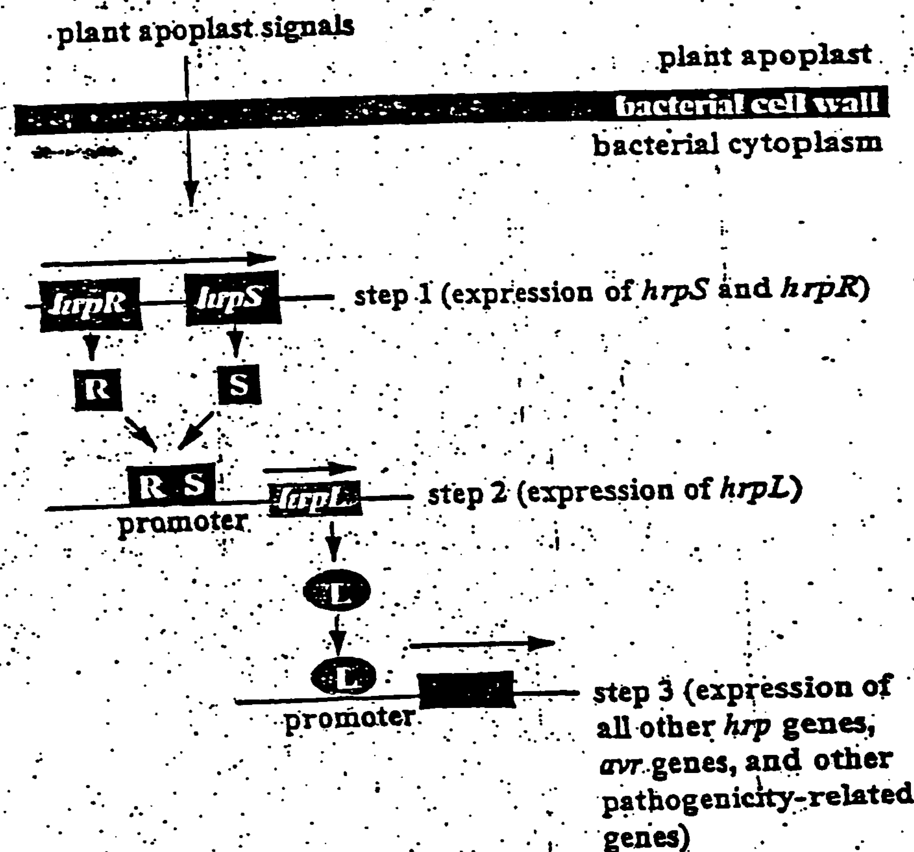

Fig. 3. Diagram of the signal transduction cascade in the detection of the plant apoplast environment by *Pseudomonas syringae*. The plant apoplast environment (limited nutrients and/or certain unique compounds) activates the expression of *hrpS* and *hrpR* by a mechanism yet to be understood (step 1). The *hrpS* and *hrpR* gene products (S and R, respectively) bind to and activate the promoter of the *hrpL* gene (step 2). The *hrpL* gene product (L), in turn, binds to promoters of other *hrp* genes, *avr* genes, and other bacterial pathogenicity-related genes to promote the expression of these genes, resulting in the initiation of diverse plant–bacteria interactions (step 3). Modified from Xiao et al. (51).

evidence seem to support this relationship. First, *hrp* genes are highly conserved among diverse plant-pathogenic bacteria, whereas virulence factors vary greatly among bacteria. Second, while mutations in the *hrp* gene completely abolish both bacterial pathogenicity and elicitation of the HR, mutations in virulence genes (e.g., toxin-production genes) often do not eliminate pathogenicity and have no effect on bacterial elicitation of the HR (3,19,49).

hrp Gene Functions and Disease Management

A major reason for discovering bacterial and plant factors critical for bacterial pathogenesis and plant resistance is to develop novel and environmentally safe strategies for controlling plant diseases. The discovery that the Hrp secretion apparatus is crucial to bacterial pathogenesis provides a foundation for designing novel chemicals and antibodies that would block the assembly of the Hrp secretion apparatus or the passage of bacterial virulence proteins through it. Alternatively, susceptible crop plants could be genetically engineered with genes encoding proteinaceous HR elicitors, such as harpins, under the control of plant promoters inducible by virulent pathogens. If this approach were successful, the HR or resistance would be triggered in otherwise compatible interactions, limiting disease development.

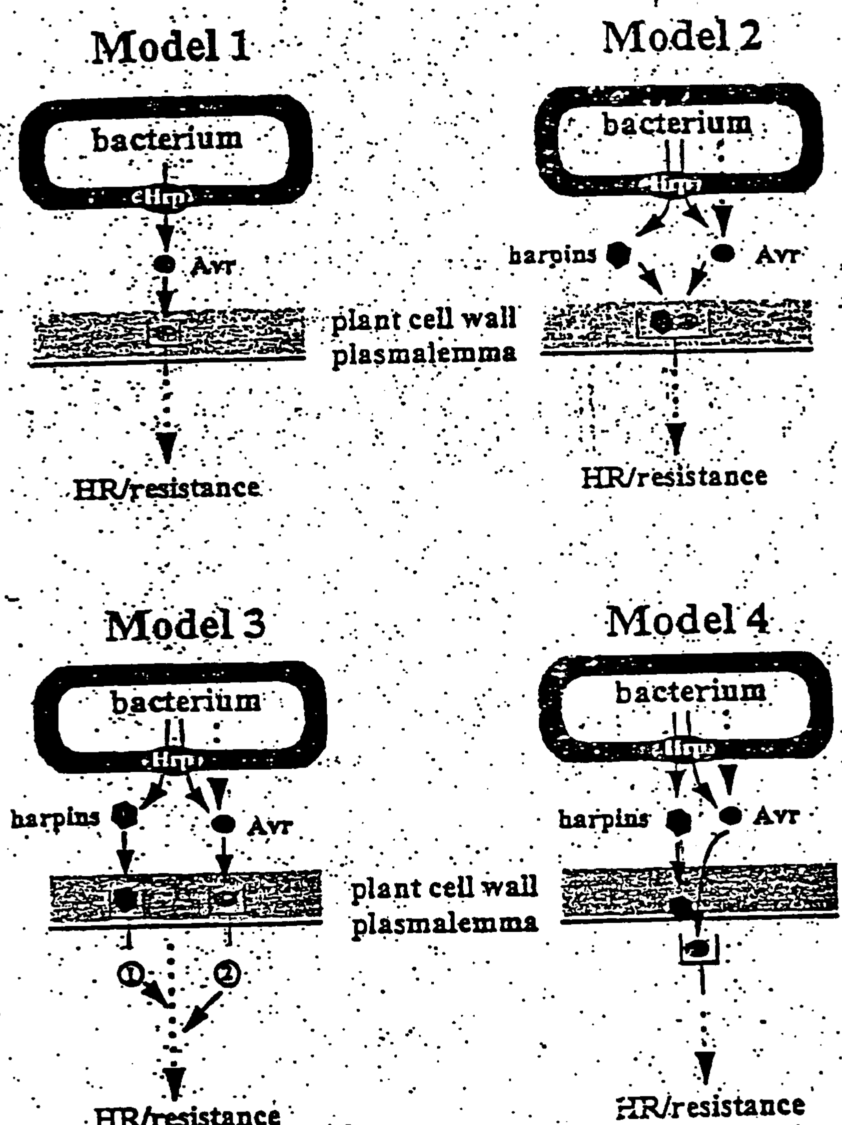

Fig. 4. Working models for possible interactions between *hrp* genes and *avr* genes. Model 1: Avr signals (Avr proteins or their enzymatic products) are secreted through the Hrp secretion apparatus to elicit the hypersensitive response (HR) and resistance. Model 2: Harpins and Avr signals modify each other before interacting with plant receptors. Avr signals may or may not be secreted via the Hrp secretion apparatus. Model 3: Harpins and Avr signals interact with respective plant receptors. Plant response elicited by harpins must precede plant response elicited by Avr signals. Avr signals may or may not be secreted via the Hrp secretion apparatus. Model 4: Avr proteins are secreted into the plant cell with the help of harpins. Avr signals may or may not be secreted via the Hrp secretion apparatus. In models 1 to 3, receptors for Avr proteins are presumed to be on the plant cell surface. In model 4, receptors for Avr proteins are inside the plant cell.

Acknowledgments

We thank Karen Bird for help in preparation of this paper, Paul Vincelli and Robert P. Scheffer for critical review, and Marlene Cameron for illustrations. Research in our laboratory is supported by grants from USDA and DOE.

Literature Cited

1. Agrios, G. N. 1988. Plant Pathology. 3rd ed. Academic Press, San Diego, CA.
2. Arlat, M., van Gijsegem, F., Huet, J. C., Pernollet, J. C., and Boucher, C. A. 1994. PopA1, a protein which induces a hypersensitivity-like response on specific Petunia genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum*. EMBO J. 13:543-553.
3. Barta, T. M., Kinscherf, T. G., and Willis, D. K. 1992. Regulation of tabtoxin production by the *lemA* gene in *Pseudomonas syringae*. J. Bacteriol. 174:3021-3029.
4. Bauer, D. W., Wei, Z.-M., Beer, S. V., and Collmer, A. 1995. *Erwinia chrysanthemi* harpin$_{Ech}$: An elicitor of the hypersensitive response that contributes to soft-rot pathogenesis. Mol. Plant-Microbe Interact. 8:484-491.
5. Beer, S. V., Bauer, D. W., Jiang, X. H., Laby, R. J., Sneath, B. J., Wei, Z., Wilcox, D. A., and Zumoff, C. H. 1990. The *hrp* gene cluster of *Erwinia amylovora*. Pages 53-60 in: Advances in Molecular Genetics of Plant-Mi-

crobe Interactions, Vol. 1. H. Hennecke and D. P. S. Verma, eds. Kluwer Academic Publishers, Dordrecht.
6. Bonas, U. 1994. *hrp* genes of phytopathogenic bacteria. Curr. Top. Microbiol. Immunol. 192:79–98.
7. Collmer, A., and Keen, N. T. 1986. The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. 24:383–409.
8. Dangl, J. L. 1995. Genes involved in bacterial pathogenesis of plants. Pages 293-304 in: Pathogenesis and Host Specificity in Plant Diseases: Histochemical, Biochemical, Genetic and Molecular Bases. Vol. I, Prokaryotes. U. S. Singh, R. P. Singh, and K. Kohmoto, eds. Pergamon, U.K.
9. Denny, T. P. 1995. Involvement of bacterial polysaccharide in plant pathogenesis. Annu. Rev. Phytopathol. 33:173–179.
10. Dixon, R. A., Harrison, M. J., and Lamb, C. J. 1994. Early events in the activation of plant defense responses. Annu. Rev. Phytopathol. 32:479-501.
11. El-Banoby, F. E., and Rudolph, K. 1979. Induction of water-soaking in plant leaves by extracellular polysaccharides from phytopathogenic pseudomonads and xanthomonads. Physiol. Plant Pathol. 15:341-349.
12. Fenselau, S., Balbo, I., and Bonas, U. 1992. Determinants of pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are related to proteins involved in secretion in bacterial pathogens of animals. Mol. Plant-Microbe Interact. 5:390-396.
13. Fett, W. F., and Dunn, M. F. 1989. Exopolysaccharides produced by phytopathogenic *Pseudomonas syringae* pathovars in infected leaves of susceptible hosts. Plant Physiol. 89:5-9.
14. Flor, A. H. 1947. Host-parasite interactions in flax rust - its genetics and other implications. Phytopathology 45:680-685.
15. Genin, S., Gough, C. L., Zischek, C., and Boucher, C. A. 1992. Evidence that the *hrpB* gene encodes a positive regulator of pathogenicity genes from *Pseudomonas solanacearum*. Mol. Microbiol. 6:3065-3076.
16. Goodman, R. N., and Novacky, A. 1994. The hypersensitive reaction in plants to pathogens: A resistance phenomenon. American Phytopathological Society, St. Paul, MN.
17. Gough, C. L., Genin, S., Zischek, C., and Boucher, C. A. 1992. *hrp* genes of *Pseudomonas solanacearum* are homologous to pathogenicity determinants of animal pathogenic bacteria and are conserved among plant pathogenic bacteria. Mol. Plant-Microbe Interact. 5:384-389.
18. Grimm, C., and Panopoulos, N. J. 1989. The predicted protein product of a pathogenicity locus from *Pseudomonas syringae* pv. *phaseolicola* is homologous to a highly conserved domain of several procaryotic regulatory proteins. J. Bacteriol. 171:5031-5038.
19. Gross, D. C. 1991. Molecular and genetic analysis of toxin production by pathovars of *Pseudomonas syringae*. Annu. Rev. Phytopathol. 29:247-278.
20. He, S. Y., Huang, H.-C., and Collmer, A. 1993. *Pseudomonas syringae* pv. *syringae* harpin$_{Pss}$: A protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. Cell 73:1255-1266.
21. He, S. Y., Lindeberg, M. L., and Collmer, A. 1993. Protein secretion by plant pathogenic bacteria. Pages 39-64 in: Biotechnological Prospects for Plant Pathogen Control. I. Chet, ed. John Wiley & Sons, New York.
22. Huang, H.-C., He, S. Y., Bauer, D. W., and Collmer, A. 1992. The *Pseudomonas syringae* pv. *syringae* 61 *hrpH* product: An envelope protein required for elicitation of the hypersensitive response in plants. J. Bacteriol. 174:6878-6885.
23. Huang, H.-C., Lin, R.-H., Chang, C.-J., Collmer, A., and Deng, W.-L. 1995. The complete *hrp* gene cluster of *Pseudomonas syringae* pv. *syringae* 61 includes two blocks of genes required for harpin$_{Pss}$ secretion that are arranged colinearly with *Yersinia ysc* homologs. Mol. Plant-Microbe Interact. 8:733-746.
24. Huang, H. C., Schuurink, R., Denny, T. P., Atkinson, M. M., Baker, C. J., Yucel, I., Hutcheson, S. W., and Collmer, A. 1988. Molecular cloning of a *Pseudomonas syringae* pv. *syringae* gene cluster that enables *Pseudomonas fluorescens* to elicit the hypersensitive response in tobacco. J. Bacteriol. 170:4748-4756.
25. Huynh, T. V., Dahlbeck, D., and Staskawicz, B. J. 1989. Bacterial blight of soybean: Regulation of a pathogen gene determining host cultivar specificity. Science 245:1374-1377.
26. Innes, R. W., Bent, A. F., Kunkel, B. N., Bisgrove, S. R., and Staskawicz, B. J. 1993. Molecular analysis of avirulence gene *avrRpt2* and identification of a putative regulatory sequence common to all known *Pseudomonas syringae* avirulence genes. J. Bacteriol. 175:4859-4869.
27. Keen, N. T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. 24:447-463.
28. Keen, N. T., Tamaki, S., Kobayashi, D., Gerhold, D., Stayton, M., Shen, H., Gold, S., Lorang, J., Thordal-Christensen, H., Dahlbeck, D., and Staskawicz, B. 1990. Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction. Mol. Plant-Microbe Interact. 3:112-121.
29. Klement, Z. 1963. Rapid detection of pathogenicity of phytopathogenic pseudomonads. Nature 199:299-300.
30. Knoop, V., Staskawicz, B., and Bonas, U. 1991. Expression of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria* is not under the control of *hrp* genes and is independent of plant factors. J. Bacteriol. 173:7142-7150.
31. Laby, R. J., and Beer, S. V. 1992. Hybridiza

Suresh Gopalan

S. Y. He

Dr. Gopalan is a research associate at the Department of Energy Plant Research Laboratory, Michigan State University. He received his B.S. degree in mechanical engineering and his M.S. degree in bioscience under the dual degree program at the Birla Institute of Technology and Science, India, in 1983. He received his Ph.D. degree in biotechnology at the Center for Biotechnology, Anna University, India, in 1994. His thesis was on the development of a biopesticide based on *Bacillus sphaericus* for mosquito control. He joined S. Y. He's laboratory in October 1993 as a postdoctoral fellow at the University of Kentucky. In 1995, he moved with He to the DOE Plant Research Laboratory. His current research focuses on molecular aspects of plant responses to bacterial pathogens.

Dr. He is an assistant professor in the Department of Energy Plant Research Laboratory at Michigan State University. He received his B.S. and M.S. degrees in plant protection at Zhejiang Agricultural University, People's Republic of China, and his Ph.D. degree in plant pathology at Cornell University. He joined the faculty of the University of Kentucky in 1993. In 1995, he moved to Michigan State University. His research interests are in molecular plant-microbe interactions.

tion and functional complementation of the *hrp* gene cluster from *Erwinia amylovora* strain Ea321 with DNA of other bacteria. Mol. Plant-Microbe Interact. 5:412-419.

32. Lamb, C. J., Lawton, M. A., Dron, M., and Dixon, R. A. 1989. Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell 56:215-224.

33. Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. 1986. Gene cluster of *Pseudomonas syringae* pv. *phaseolicola* controls pathogenicity of bean plants and hypersensitivity on nonhost plants. J. Bacteriol. 168:512-522.

34. Martin, G. B., Brommonschenkel, S. H., Chunwongse, J., Frary, A., Ganal, M. W., Spivey, R., Wu, T., Earle, E. D., and Tanksley, S. D. 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432-1436.

35. Niepold, F., Anderson, D., and Mills, D. 1985. Cloning determinants of pathogenesis from *Pseudomonas syringae* pathovar *syringae*. Proc. Natl. Acad. Sci. USA 82:406-410.

36. Preston, G., Huang, H.-C., He, S. Y., and Collmer, A. 1995. The HrpZ proteins of *Pseudomonas syringae* pvs. *syringae*, *glycinea*, and *tomato* are encoded by an operon containing *Yersinia ysc* homologs and elicit the hypersensitive response in tomato but not in soybean. Mol. Plant-Microbe Interact. 8:717-732.

37. Rahme, L. G., Mindrinos, M. N., and Panopoulos, N. J. 1992. Plant and environmental sensory signals control the expression of *hrp* genes in *Pseudomonas syringae* pv. *phaseolicola*. J. Bacteriol. 174:3499-3507.

38. Salmeron, J. M., and Staskawicz, B. J. 1993. Molecular characterization and *hrp* dependence of the avirulence gene *avrPto* from *Pseudomonas syringae* pv. *tomato*. Mol. Gen. Genet. 239:6-16.

39. Salmond, G. C. 1994. Secretion of extracellular virulence factors by plant pathogenic bacteria. Annu. Rev. Phytopathol. 32:181-200.

40. Shen, H., and Keen, N. T. 1993. Characterization of the promoter of avirulence gene D from *Pseudomonas syringae* pv. *tomato*. J. Bacteriol. 175:5916-5924.

41. Shulte, R., and Bonas, U. 1992. A *Xanthomonas* pathogenicity locus is induced by sucrose and sulfur-containing amino acids. Plant Cell 4:79-86.

42. Silverstone, S. E., Gilchrist, D. G., Bostock, R. M., and Kosuge, T. 1993. The 73-kb pIAA plasmid increases competitive fitness of *Pseudomonas syringae* subspecies *savastanoi* in oleander. Curr. Plant Sci. Biotechnol. Agric. 14:659-664.

43. Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G., and Jones, J. D. G. 1995. Molecular genetics of plant disease resistance. Science 268:661-667.

44. Staskawicz, B. J., Dahlbeck, D., and Keen, N. T. 1984. Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr. Proc. Natl. Acad. Sci. USA 81:6024-6028.

45. Van Gijsegem, F., Genin, S., and Boucher, C. 1995. *hrp* and *avr* genes, key determinants controlling the interactions between plants and Gram-negative phytopathogenic bacteria. Pages 273-292 in: Pathogenesis and Host Specificity in Plant Diseases: Histochemical, Biochemical, Genetic and Molecular Bases. Vol. 1, Prokaryotes. U. S. Singh, R. P. Singh, and K. Kohmoto, eds. Pergamon, U.K.

46. Van Gijsegem, F., Gough, C., Zischek, C., Niqueux, E., Arlat, M., Genin, S., Barberis, P., German, S., Castello, P., and Boucher, C. 1995. The *hrp* gene locus of *Pseudomonas solanacearum*, which controls the production of a type III secretion system, encodes eight proteins related to components of the bacterial flagellar biogenesis complex. Mol. Microbiol. 15:1095-1114.

47. Wei, Z.-M., Laby, R. J., Zumoff, C. H., Bauer, D. W., He, S. Y., Collmer, A., and Beer, S. V. 1992. Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science 257:85-88.

48. Wei, Z.-M., Sneath, B. J., and Beer, S. V. 1992. Expression of *Erwinia amylovora hrp* genes in response to environmental stimuli. J. Bacteriol. 174:1875-1882.

49. Willis, D. K., Barta, T. M., and Kinscherf, T. G. 1991. Genetics of toxin production and resistance in phytopathogenic bacteria. Experientia 47:765-771.

50. Willis, D. K., Rich, J. J., and Hrabak, E. M. 1991. *hrp* genes of phytopathogenic bacteria. Mol. Plant-Microbe Interact. 4:132-138.

51. Xiao, Y., Heu, S., Yi, J., Lu, Y., and Hutcheson, S. W. 1994. Identification of a putative alternative sigma factor and characterization of a multicomponent regulatory cascade controlling the expression of *Pseudomonas syringae* pv. *syringae* Pss61 *hrp* and *hrmA* genes. J. Bacteriol. 176:1025-1036.

52. Xiao, Y., Lu, Y., Heu, S., and Hutcheson, S. W. 1992. Organization and environmental regulation of the *Pseudomonas syringae* pv. *syringae* 61 *hrp* cluster. J. Bacteriol. 174:1734-1741.

53. Yucel, I., Xiao, Y., and Hutcheson, S. V. 1989. Influence of *Pseudomonas syringae* culture conditions on initiation of the hypersensitive response of cultured tobacco cells. Appl. Environ. Microbiol. 55:1724-1729.